

Serial No. 09/981,648

IN THE CLAIMS

1 24. (previously added) A method for making an infectious adenovirus which comprises
2 contacting a cell with or introducing into a cell:

3 a. a first nucleic acid sequence being a plasmid comprising a circularized adenovirus
4 DNA molecule having a deletion of an adenoviral packaging signal, and which, by itself, in the
5 absence of intermolecular recombination, is incapable of generating an infectious, packageable
6 adenovirus; and

7 b. a second nucleic acid sequence which, by itself, in the absence of intermolecular
8 recombination, is incapable of generating an infectious, packageable adenovirus, and encoding
9 adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or
10 intermolecular recombination with said first nucleic acid sequence, are incapable of encoding an
11 infectious, packageable adenovirus;

12 provided that said first and said second nucleic acid sequences each comprise a head-to-head ITR
13 junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous
14 recombination may occur between said first and said second nucleic acid sequences, whereby
15 said first and said second nucleic acids recombine to form said infectious adenovirus.

1 25. (previously added) The method according to claim 24 wherein said adenovirus DNA
2 additionally comprises at least one of (i) a deletion of, or (ii) a modification in, an adenoviral
3 gene selected from the group consisting of adenoviral F1 sequences 3' of said packaging signal,
4 adenoviral fibre gene sequences, adenoviral E3 gene sequences, and adenoviral E4 gene
5 sequences.

1 26. (previously added) A method for making an infectious adenovirus which comprises
2 contacting a cell with or introducing into a cell:

3 a. a first nucleic acid sequence encoding adenovirus sequences and which, by itself, in
4 the absence of intermolecular recombination, is incapable of generating an infectious,

Serial No. : 09/981,648

5 packageable adenovirus; and

6 b. a second nucleic acid sequence, which is a plasmid formed by combination of (i) at
7 least one of the shuttle plasmids selected from the group consisting of pDC111, pDC112,
8 pDC113, pDC114, pDC115, pDC116, pDC117, and pDC118, and (ii) a polycloning site or a
9 foreign DNA or an expression cassette, and which second nucleic acid sequence, by itself, in the
10 absence of intermolecular recombination, is incapable of generating an infectious, packageable
11 adenovirus;

12 provided that said first and said second nucleic acid sequences each comprise a head-to-head ITR
13 junction and sufficient overlapping adenoviral nucleic acid sequences such that homologous
14 recombination may occur between said first and said second nucleic acid sequences, whereby
15 said first and said second nucleic acids recombine to form said infectious adenovirus.

1 27. (previously added) A recombinant adenovirus vector system comprising:

2 a. a first nucleic acid sequence encoding adenovirus sequences and which, by itself,
3 in the absence of intermolecular recombination, is incapable of generating an
4 infectious, packageable adenovirus, said first nucleic acid sequence comprising a
5 head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid
6 sequences such that homologous recombination with homologous sequences in a
7 second nucleic acid sequence may occur; and

8 b. the second nucleic acid sequence, which is a plasmid formed by combination of
9 (i) at least one of the shuttle plasmids selected from the group consisting of
10 pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, and pDC118,
11 and (ii) a polycloning site or a foreign DNA or an expression cassette, and which
12 second nucleic acid sequence, by itself, in the absence of intermolecular
13 recombination, is incapable of generating an infectious, packageable adenovirus;
14 said second nucleic acid sequence comprising a head-to-head ITR junction and
15 sufficient overlapping adenoviral nucleic acid sequences to permit homologous
16 recombination with said first nucleic acid sequence;

Serial No. : 09/981,648

17 whereby said first and said second nucleic acids homologously recombine in a cell to
18 form said infectious adenovirus.

1 28. (previously added) A recombinant adenovirus vector system comprising:
2 a. a first nucleic acid sequence encoding adenovirus sequences, and which, by itself,
3 in the absence of intermolecular recombination, is incapable of generating an
4 infectious, packageable adenovirus, said first nucleic acid sequence comprising a
5 head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid
6 sequences such that homologous recombination with homologous sequences in a
7 second nucleic acid sequence may occur; and
8 b. the second nucleic acid sequence which, by itself, in the absence of
9 intermolecular recombination, is incapable of generating an infectious,
10 packageable adenovirus; said second nucleic acid sequence comprising a head-to-
11 head ITR junction, an adenoviral packaging signal, and sufficient overlapping
12 adenoviral nucleic acid sequences to permit homologous recombination with said
13 first nucleic acid sequence;
14 whereby said first and said second nucleic acids homologously recombine in a cell to
form said infectious, packageable adenovirus, and wherein said cell expresses adenoviral
E1.

1 29. (amended herein) A kit for construction of recombinant adenovirus vectors comprising:
2 (A) a first nucleic acid sequence encoding adenovirus sequences and which, by itself,
3 in the absence of intermolecular recombination, is incapable of generating an
4 infectious, packageable adenovirus, and said first nucleic acid sequence
5 comprising a head-to-[tail]head ITR junction and sufficient adenoviral sequences
6 to permit homologous recombination with similar sequences in a second nucleic
7 acid sequence;
8 (B) the second nucleic acid sequence which is a plasmid formed by combination of (i)

Serial No. : 09/981,648

9 at least one of the shuttle plasmids selected from the group consisting of pDC111,
10 pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, and pDC118, and (ii) a
11 polycloning site or a foreign DNA or an expression cassette, and which, by itself,
12 in the absence of intermolecular recombination, is incapable of generating an
13 infectious, packageable adenovirus, and said second nucleic acid sequence
14 comprising a head-to-head ITR junction and sufficient adenoviral sequences to
15 permit homologous recombination with similar sequences in said first nucleic
16 acid; and

17 (C) a cell wherein, when said component (A) and said component (B) are
18 cotransfected and recombined through homologous recombination, an infectious
19 recombinant adenovirus vector is produced.

1 30. (previously added) A recombinant adenovirus vector system comprising:
2 a. a first nucleic acid sequence comprising a deletion in the adenoviral fibre gene,
3 and encoding other adenovirus sequences, and which, by itself, in the absence of
4 intermolecular recombination, is incapable of generating an infectious,
5 packageable adenovirus, said first nucleic acid sequence comprising a head-to-
6 head ITR junction and sufficient overlapping adenoviral nucleic acid sequences
7 such that homologous recombination with homologous sequences in a second
8 nucleic acid sequence may occur; and
9 b. the second nucleic acid sequence which, by itself, in the absence of
10 intermolecular recombination, is incapable of generating an infectious,
11 packageable adenovirus; said second nucleic acid sequence comprising a head-to-
12 head ITR junction, an adenoviral packaging signal, and sufficient overlapping
13 adenoviral nucleic acid sequences to permit homologous recombination with said
14 first nucleic acid sequence;
15 whereby said first and said second nucleic acids homologously recombine in a cell to
16 form said infectious, packageable adenovirus.

Serial No. : 09/981,648

- 1 31. (previously added) A recombinant adenovirus vector system comprising:
- 2 a. a first nucleic acid sequence encoding adenovirus sequences and which, by itself,
- 3 in the absence of intermolecular recombination, is incapable of generating an
- 4 infectious, packageable adenovirus, said first nucleic acid sequence comprising a
- 5 head-to-head ITR junction and sufficient overlapping adenoviral nucleic acid
- 6 sequences such that homologous recombination with homologous sequences in a
- 7 second nucleic acid sequence may occur; and
- 8 b. the second nucleic acid sequence which, by itself, in the absence of
- 9 intermolecular recombination, is incapable of generating an infectious,
- 10 packageable adenovirus; said second nucleic acid sequence comprising a head-to-
- 11 head ITR junction, an adenoviral packaging signal, an adenoviral gene mutation,
- 12 and sufficient overlapping adenoviral nucleic acid sequences to permit
- 13 homologous recombination with said first nucleic acid sequence;
- 14 whereby said first and said second nucleic acids homologously recombine in a cell to
- 15 form said infectious, packageable adenovirus, and wherein said adenoviral gene mutation
- is rescued into said infectious, packageable adenovirus.

- 1 32. (previously added) The recombinant adenovirus vector system according to claim 31
- 2 wherein said adenoviral gene mutation rescued into said adenoviral vector recombinant is
- 3 comprised of at least one of a mutation in the adenoviral fibre gene, a mutation in the
- 4 adenoviral E4 gene, and a mutation in the adenoviral E3 gene.